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10/561,041	07/03/2006	Atsushi Miyawaki	P28993	3770

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EXAMINER	
BRADLEY, CHRISTINA	
ART UNIT	**PAPER NUMBER**
1654	

NOTIFICATION DATE	DELIVERY MODE
12/23/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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ADVISORY ACTION

Status of the Claims

1. Claims 1-37 are pending. Claims 1-17 and 20-35 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 01/08/2010.

2. Claims 18, 19, 36 and 37 were amended in the response filed 12/06/2010 to cancel SEQ ID NOs: 7 and 8. The amendment is entered.

Interview Summary

3. In a telephone interview with Walter Schlapkohl on 12/14/2010, the Examiner proposed an amendment to limit claim 18 to an isolated DNA encoding a fluorescence protein comprising SEQ ID NO: 5 or 7, and to limit claim 19 to an isolated DNA comprising SEQ ID NO: 6 or 8, which encodes a fluorescent protein. The proposed amendment would place the case in condition for allowance. The Examiner indicated that the claim amendment and arguments filed 12/06/2010 were insufficient to overcome the rejections on record. In a follow-up conversation on 12/17/2010, Mr. Schlapkohl indicated that Applicant has not determined whether to accept or reject the Examiner's proposal. As a result, this Advisory Action is issued. Applicant is invited upon receipt of this Action to consider the proposed amendment and to contact the Examiner to discuss prosecution of the case prior to issuing a response.

Claim Rejections - 35 USC § 112

4. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it

pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 18, 19, 36 and 37 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. MPEP § 2163 states that the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus.

6. In the response filed 12/07/2010 Applicant traversed the rejection by providing the Examiner with a copy of the USPTO's Written Description Training Materials (Revision 1, March 25, 2008). The Examiner has previously considered each of the factors cited therein has concluded that the claims fail to comply with the written description requirement of 35 U.S.C. 112, first paragraph. In response to Applicant's arguments and to clarify the Examiner's rationale with respect to each factor, the following analysis is presented.

Factor 1: A determination as to what the claim as a whole covers. In making this determination, the examiner should consider and discuss the full scope of the claim.

7. Claim 18 is drawn to DNA encoding a fluorescent protein comprising SEQ ID NO: 5, or a fluorescent protein with one to ten substitutions, deletions or additions relative to SEQ ID NO: 5. Claim 19 is drawn to DNA comprising SEQ ID NO: 6, which encodes SEQ ID NO: 5, or

DNA with one to thirty substitutions, deletions or additions relative to SEQ ID NO: 6, which encodes a fluorescent protein.

8. The genus is characterized by both structural and functional limitations. With respect to structure, although a claim limitation requiring greater than 95.6% identity may appear limited, the number of amino acid sequences comprising a deletion, substitution and/or addition of one to ten amino acids with respect to SEQ ID NO: 5 is actually enormous. SEQ ID NO: 5 is a set of n=232 elements or amino acid residues. The number of species that meet the structural limitations of the genus is related to the number of combinations of one to ten residues (k) where the substitution, addition or deletion occurs and the number of possibilities for each k-combination. The number of k-combinations from a given set of n elements is:

$$\binom{n}{k} = \frac{n!}{k!(n-k)!},$$

(see Weisstein, Eric W. "Combination." From MathWorld--A Wolfram Web Resource. http://mathworld.wolfram.com/Combination.html). The number of possibilities for each combination, assuming substitution to one of the other 19 natural amino acids, a deletion or the addition of one of the 20 natural amino acids, is 40^k. Applying the formula above to the instant claim, the number of combinations of k=10 substituted amino acids for the 232 residue sequence is 232!/(10!*222!). At each of the 10 positions there are 40 possibilities, yielding 40^{10} possibilities for each k=10 combination. Therefore, the total number of sequences included in the sub-genus genus in which ten amino acids of SEQ ID NO: 5 are substituted with a naturally occurring amino acid or deleted or where one of the 20 natural amino acids is added is (232!/(10!*222!)) x 40^{10} or 1.07 x 10^{33}. For the sub-genus in which one amino acid is

substituted with a naturally occurring amino acid or deleted or where one of the 20 natural amino acids is added, there are (232!/(1!*231!)) x 40^1 or 9280 possible sequences. For the sub-genus in which two amino acids are substituted with a naturally occurring amino acid or deleted or where one of the 20 natural amino acids is added, there are (232!/(2!*230!)) x 40^2 or 42,873,600 possible sequences. Given that methods for unnatural amino acid mutagenesis are known in the art, the number of species that meet the structural limits of the claims are even larger.

9. Only those sequences meeting the structural and functional requirements of the genus are encompassed by the claim. Therefore, the claim encompasses all of the sequences meeting the structural requirements that are also fluorescent. Although with the aid of a computer it may be possible to determine the amino acid sequences of the proteins that meet the structural requirements of the claim (i.e. one to ten substitutions, deletions or additions relative to SEQ ID NO: 5), it is not readily apparent from the claims or the specification which of these sequences are fluorescent.

10. Therefore, to meet the written description requirement of 35 U.S.C. § 112, first paragraph, the specification must disclose a representative number of species that meet both the structural and functional limitations of the genus or the specification and/or the prior art must identify the structural elements that correlate to the claimed function in a manner that demonstrates to one of ordinary skill in the art that Applicant was in possession of the claimed genus at the time the application was filed. In the instant case, the specification and/or the prior art must establish which of the enormous number of protein sequences that satisfy the structural limitations of the claim are also fluorescent.

Factor 2. A full review of the application to understand how the applicant provides support for the claimed invention including each element and/or step. This review includes comparing the claim scope with the scope of the description.

11. The original specification disclosed two embodiments of the invention: SEQ ID NO: 5 and a variant in which glutamine at position 166 was substituted with histidine, SEQ ID NO: 7 (encoded by SEQ ID NOs: 6 and 8, respectively). SEQ ID NO: 5 was cloned from Acropora Sp. and was shown to have an excitation maximum wavelength of 472 nm, a fluorescent emission maximum wavelength of 496 nm, a molar extinction coefficient of 27,250, a quantum yield of 0.9 and a pKa of 6.6 (Table 3). SEQ ID NO: 7 has a single amino substitution relative to SEQ ID NO: 5, Q166H, and exhibits an excitation maximum wavelength of 462 nm, a fluorescent emission maximum wavelength of 493 nm and a pKa of 5.6 (p. 49). The specification does not disclose variants of SEQ ID NO: 5 wherein amino acids at other positions are modified nor does it disclose variants of SEQ ID NO: 5 wherein position 166 is modified in a different manner. As discussed above the claim scope is potentially enormous depending on how many of the sequences that meet the structural requirements are also fluorescent; in comparison, the scope of the description which only includes two species, is extremely narrow. Furthermore, the specification fails to disclose sufficient identifying characteristics of the genus, which is discussed in more detail below.

Factor 3: A determination as to whether one skilled in the art would recognize that the applicant was in possession of the claimed invention as a whole at the time of filing. This determination should include the following considerations:

a) Actual Reduction to Practice

12. Two embodiment of the invention were reduced to practice at the time the Application was filed. SEQ ID NO: 5 was cloned from Acropora Sp. and was shown to have an excitation maximum wavelength of 472 nm, a fluorescent emission maximum wavelength of 496 nm, a molar extinction coefficient of 27,250, a quantum yield of 0.9 and a pKa of 6.6 (Table 3). SEQ ID NO: 7 has a single amino substitution relative to SEQ ID NO: 5, Q166H, and exhibits an excitation maximum wavelength of 462 nm, a fluorescent emission maximum wavelength of 493 nm and a pKa of 5.6 (p. 49). The specification does not disclose variants of SEQ ID NO: 5 wherein amino acids at other positions are modified nor does it disclose variants of SEQ ID NO: 5 wherein position 166 is modified in a different manner. It is not possible to discern from the data on these two proteins alone, which of the countless combinations of one to ten substitutions, deletions and additions relative to SEQ ID NO: 5 will preserve the fluorescence activity of the protein.

b) Disclosure of drawings or structural chemical formulas

13. Figures 4 and 5 show the excitation and emission spectra and the pH sensitivity of SEQ ID NOs: 5 and 7, referred to as MICy and MiCy2, respectively. From this experimental data and the discussion thereof on p. 49 of the specification, it is clear that substituting Gln for His at position 166 of SEQ ID NO: 5 preserves the fluorescence activity of the specification. The structural modification results in a blue shift of 10 nm for the excitation maximum, a blue shift of 3 nm for the emission maximum, and a shift in the pH sensitivity. Given the breadth of the claims, these drawings are not representative of the full scope of the genus. The emission and excitation spectra from a protein and a single variant thereof are not sufficient to permit one of ordinary skill in the art to predict the effect of any other substitution, deletion or addition on the

protein fluorescence. The specification does not include other drawings that would be useful for describing the claimed genus such a structure of the chromophore, an alignment of the proteins to show homology to related fluorescent proteins, or a three dimensional structure of the protein or related proteins to help correlate the various amino acid positions to the chromophore, the residues in close proximity to the chromophore and to the overall fold of the protein.

c) Sufficient relevant identifying characteristic

i. Complete structure: As stated above, the complete structure of two species, SEQ ID NOs: 5 and 7, is disclosed.

ii. Partial structure: The specification does not disclose a partial structure of a fluorescent protein that meets the structural requirements of the genus. Although from the statement "a protein comprising from one to ten substitutions, deletions or additions relative to SEQ ID NO: 5" one of ordinary skill in the art could determine if a given amino acid sequence meets the structural requirements of the genus, it would not be possible to determine from the sequence alone if the protein is fluorescent. Unless all sequences having one to ten substitutions, deletions or additions relative to SEQ ID NO: 5 are fluorescent, this description alone does not constitute a partial structure for the genus. Furthermore, the specification does not disclose a partial structure such as the chromophore structure, or the three dimensional structure of the region in close proximity to the chromophore.

iii. Physical and/or chemical properties: The excitation and emission spectra of SEQ ID NOs: 5 and 7 and the pH sensitivity of the excitation maximum presented in the specification raise more questions about the physical properties of the genus than they answer. The claims require that the proteins be generally fluorescent not that they have a particular fluorescence

profile. Yet the data on SEQ ID NOs: 5 and 7 suggest only that substituting His for Gln at position 166 will preserve the fluorescence of SEQ ID NO: 5; it does not suggest the physical basis for the fluorescence activity in its entirety and therefore does not describe which additional substitutions, deletions or additions could be made. The specification states that the Q166H substitution alters the pH sensitivity of the excitation at the wavelength of maximum absorption for each protein but it does not explore this effect with experimentation or even suggest a physical basis for this phenomenon. The protonation state of the amino acid side chain at position 166 has an impact on the absorption intensity at the excitation maximum but it is not clear from the specification why. Therefore, one of ordinary skill in the art can not extrapolate from the pH sensitivity data on the two proteins what the physical role of position 166 in determining fluorescence is. It is not clear if position 166 is part of the chromophore, if it influences chromophore formation indirectly, if it contacts the chromophore in the folded protein, or if it has a role in the overall stability and dynamics of the folded protein such that when it is altered the environment around the chromophore is impacted. Additionally, the limited data in the specification does not speak to the role of the other 231 amino acids in fluorescence. The specification therefore does not therefore establish the physical basis for fluorescence in the claim protein genus. Understanding the physical basis for fluorescence is critical to determining which of the sequences that meet the structural requirements of the genus also meet the functional requirements of the genus.

iv. Functional characteristics when coupled with a known or disclosed correlation between function and structure: The specification does not describe a general correlation between structure and function for the claimed genus. The role of the 232 amino acids of SEQ

ID NO: 5 in the chromophore, chromophore formation, chromophore environment and overall fold, stability and dynamics of the protein are not described. As a result, it is impossible to predict, based on the specification, how changing any position other than the single substitution of SEQ ID NO: 7, will have on fluorescence.

d) Method of making the claim invention

14. Solid state peptide synthesis and the cloning, recombinant expression and purification of proteins is well-known in the art. It is not disputed that one of ordinary skill in the art could isolate, albeit with route experimentation and optimization, a fluorescent protein of a given sequence provided that the sequence is known. Where the specification fails to provide description is in the structure of the protein to make. For all of the reasons presented above, one of ordinary skill in the art would not know which of the countless proteins that meet the structural requirements of the claims would also be fluorescent. Thus, while it may be trivial to use commercially-available site-directed mutagenesis kits to make a protein with one to ten substitutions, deletions or additions relative to SEQ ID NO: 5, it would not be possible based on the specification to make a protein that is necessarily fluorescent. This is not an issue of enablement. Given the screening methods known in the art and in the specification, it would not be undue burden of experimentation to use error-prone PCR or DNA shuffling and screen colonies for fluorescence to make the proteins of the claimed genus. This is an issue of written description. The specification does not make clear which proteins are in the genus and which are not because it does not describe the physical basis for the claimed activity, fluorescence. In other words, the specification does not describe which proteins to make.

e. Level of skill and knowledge in the art

15. In a 2004 review, Verkhusha et al. describe the molecular properties of Anthozoa fluorescent proteins and chromoproteins (Nature Biotechnology, **2004**, 22, 289-296). GFP-like proteins are a family of homologous 25–30 kDa polypeptides that, together with A. victoria GFP mutants, cover the emission range from 442–645 nm. In comparison to other natural pigments, GFP-like proteins are unusual in that they can form internal chromophores without requiring accessory cofactors, external enzymatic catalysis or substrates other than molecular oxygen.

16. GFP-like proteins have tremendous color variety, which can be classified into four main groups: GFPs, yellow FPs (YFPs), red FPs (RFPs) and nonfluorescent CPs of different hues, from orange to blue.

17. Among known FPs, GFPs are the most abundant, as green fluorescence can be detected in the majority of Anthozoa species. GFPs are characterized by emission spectra that peak at 480–520 nm. Their excitation curves can possess either a single peak at 440–510 nm or two peaks at approximately 400 and 470–490 nm. Thus far, a single YFP has been isolated, zoanYFP (zFP538) from Zoanthus sp., which shows excitation-emission maxima at 528 nm and 538 nm, respectively. RFPs possess emission maxima at wavelengths greater than 570 nm. Often, these proteins go through a green fluorescence–emitting stage during their maturation. RFPs can be subdivided into two subgroups. The first subgroup is represented by drFP5833 (commercial name DsRed), the most popular and well-studied RFP to date. These RFPs are characterized by a broad (spectral width about 50–60 nm) emission spectrum that peaks at 570–610 nm. The second RFP subgroup is characterized by its need for UV or violet light irradiation for red chromophore formation. In the dark, these proteins mature to a GFP, whereas UV-violet irradiation causes

their fast transformation into RFP. The resulting red emission spectra are rather narrow (spectral width about 25 nm) and have a pronounced shoulder at about 630 nm.

18. Coral also possess GFP-like proteins that are CPs, which effectively absorb but practically do not emit light. Known CPs possess single absorption maxima at 560–590 nm. The wavelength of the absorption maximum determines the particular CP color; one can see soft hues of purple, crimson, lilac and almost-blue colors. In some CPs, extremely weak (quantum yield <0.001) red and far-red fluorescence can also be detected.

19. Verkhusha et al. make clear that amongst proteins sharing structural homology to GFP there is a subset of proteins that are not fluorescent, CPs. In other words, the knowledge in the art establishes that structural homology to GFP and the GFP-like family of proteins does not necessarily translate to fluorescence.

20. Verkhusha et al. offer two explanations for the color differences among GFP-like proteins: 1) different colors could arise from distinct noncovalent interactions of the chromophore with its microenvironment; and 2) chemically distinct chromophores can determine drastic spectral shifts. Verkhusha et al. go on to say that the extremely well-characterized A. victoria GFP mutants exhibit different colors primarily do to the first explanation. That is, distinct noncolavent interactions of the chromophore with the residues constituting its microenvironment lead to different spectral properties. In contrast, for Anthozoa proteins, Verkhusha et al. note that an unexpected diversity of chromophore structures has been found, suggesting the second possibility. Verkhusha et al. discuss examples of several other distinct chromophore structures in coral FPs and CPs.

21. Therefore, Verkhusha et al. makes it clear that all of the structure-activity work done on A. victoria GFP does complete the story for the physical basis for the spectral properties of coral GFP-like proteins. It is noted that instantly claimed SEQ ID NO: 5 is a coral protein.

f. Predictability in the art

22. Verkhusha et al. discuss that many drawbacks to Anthozoa fluorescence proteins such as slow folding rate and a tendency to form high molecular weight aggregates. Random mutagenesis has been applied successfully to generate fast maturing and non-oligomerizing variants. The fact that random mutagenesis techniques rather than structure based design has been utilized to alter the properties of these proteins underscores the point that the structure-function correlation for proteins is complex and unpredictable.

Factor 4: For each claim drawn to a single embodiment or species, consider the above factors in regard to that embodiment or species to determine whether one of ordinary skill in the art would recognize that the applicant was in possession of the species of embodiment at the time of filing.

23. The claim listing filed 12/06/2010 does not include claims drawn to a single embodiment or species. The claim amendment proposed by the Examiner on 12/14/2010 which limits the scope to DNA encoding SEQ ID NOs: 5 and 7, including SEQ ID NOs: 6 and 8, is fully supported by the original specification.

Factor 5: For each claim drawn to a genus, consider each of the above factors to determine whether there is disclosure of a representative number of species which would lead one skilled in the art to conclude that the applicant was in possession of the claimed invention. The number of species required to represent a genus will vary, depending on the level of skill and knowledge in the art and the variability among the claimed genus. For instance, fewer species will be required

where the skill and knowledge in the art is high, and more species will be required where the claimed genus is highly variable.

24. Each of the factors have been considered with respect to the scope of the genus throughout the analysis above. In the response filed 12/06/2010, Applicants characterize the level of ordinary skill in the art as high and remark that fluorescent proteins are generally well-known and have well-characterize structures. Accordingly, Applicant argues that the disclosure of two species is sufficient to fully support the claimed genus. The Examiner disagrees with this interpretation. There is a large body of work in the GFP-like protein art and yet there is a high level of unpredictability and complexity associated with the structural basis for the diverse array of spectral properties exhibited by these proteins. Attempts to design new GFP-like proteins with improved properties have been successful but have relied at least in part on random mutagenesis techniques, underscoring the difficulty associated with predicting how to alter a sequence in a manner that will produce a specific desired functional effect. Most importantly, the prior art does not present clear rules to distinguish GFP-like proteins that are fluorescent from those that are not fluorescent. Proteins containing a high degree of homology to GFP may be either fluorescent or non-fluorescent and it is not entirely clear why. Applicant has not addressed this issue in the specification or the arguments. For these reasons, the skilled artisan would not reasonably conclude that the inventor(s), at the time the application was filed, had possession of the full scope of the claimed invention.

25. Finally, in the response filed 12/06/2010, Applicants provide an attachment with data for SEQ ID NO: 5 and variants thereof including SEQ ID NO: 7. Applicants submit that the attached data further evidence that Applicants were in possession of the claimed subject matter at

the time the application was filed. This data is insufficient to overcome the rejection because it is not established that Applicant was in possession of the data at the time of filing or if this data was generated post-filing.

26. In conclusion, only isolated DNA encoding SEQ ID NOs: 5 and 7, including SEQ ID NOs: 6 and 8, satisfy the written description requirements of 35 U.S.C. 112, first paragraph.

Claim Rejections - 35 USC § 102

27. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

28. Claims 18, 19, 36 and 37 are rejected under 35 U.S.C. 102(a) as being anticipated by Karasawa et al. ("Cyan-emitting and orange-emitting fluorescent proteins as a donor/acceptor pair for fluorescence resonance energy transfer." Biochem. J., published 5 April 2004, 381, 307-312), as evidenced by GenBank: AB128822.1. Karasawa et al. teach a nucleotide that is 100% identical to instant SEQ ID NO: 6, which encodes an amino acid sequence that is 100% identical to instant SEQ ID NO: 5, as evidenced by GenBank AB128822.1. The protein is a fluorescent protein.

29. Applicant cannot rely upon the foreign priority papers to overcome this rejection because a translation of said papers has not been made of record in accordance with 37 CFR 1.55. See MPEP § 201.15. The translation of Application No. 2003-170326 filed 07/15/2010 is

insufficient to overcome the rejection because said application does not support the full scope of claims 18, 19, 36 and 37. Application No. 2003-170326 teaches a fluorescent protein consisting of instant SEQ ID NO: 5 but does not support the full scope of the genus for the reasons set forth above in the rejection under 35 U.S.C. 112, first paragraph.

Conclusion

30. No claims are allowed.

31. Any inquiry concerning this communication or earlier communications from the examiner should be directed to CHRISTINA BRADLEY whose telephone number is (571)272-9044. The examiner can normally be reached on Monday, Tuesday, Thursday and Friday 8:30 A.M. to 4:30 P.M.

32. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia Tsang can be reached on (571) 272-0562. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

33. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Christina Marchetti Bradley/

Primary Examiner, Art Unit 1654

cmb